



(12) **EUROPEAN PATENT APPLICATION**

(43) Date of publication:
29.08.2001 Bulletin 2001/35

(51) Int Cl.7: **A61L 27/36**, **A61K 35/32**,
A61L 24/00

(21) Application number: **00301370.3**

(22) Date of filing: **22.02.2000**

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU
MC NL PT SE
Designated Extension States:
AL LT LV MK RO SI

(72) Inventors:
• **Gertzman, Arthur A.**
New York 10980 (US)
• **Sunwoo, Moon Hae**
New Jersey 07675 (US)

(71) Applicant: **Musculoskeletal Transplant**
Foundation
Edison, New Jersey 08837-9947 (US)

(74) Representative: **W.P. Thompson & Co.**
Coopers Building,
Church Street
Liverpool L1 3AB (GB)

(54) **Malleable paste for filling bone defects**

(57) The invention is directed toward a malleable bone putty and a flowable gel composition for application to a bone defect site to promote new bone growth at the site which comprises a new bone growth inducing compound of demineralized lyophilized allograft bone powder. The bone powder has a particle size ranging from about 100 to about 850 microns and is mixed in a

high molecular weight hydrogel carrier, the hydrogel component of the carrier ranging from 0.75% to 4.5% of the composition and having a molecular weight of about at least 10,000 Daltons. The composition contains about 25% to about 35% bone e powder and can be additionally provided with BMP's and a sodium chloride based phosphate buffer.

Description**FIELD OF INVENTION**

- 5 [0001] The present invention is generally directed toward a surgical bone product and more specifically is a flowable gel and a malleable putty based on demineralized allograft bone particles mixed in a fluid carrier comprising a high molecular weight viscous excipient derived from the class of biomaterials known as hydrogels.

BACKGROUND OF THE INVENTION

10

[0002] Malleable putty is used to correct surgical defects that may be caused by trauma, pathological disease, surgical intervention or other situations where defects need to be managed in osseous surgery. It is important to have the defect filler in the form of a stable, viscous putty to facilitate the placement of the bone growth medium into the surgical site which is usually uneven in shape and depth. The surgeon will take the putty on a spatula or other instrument and

15

trowel it into the site or take it in his/her fingers to shape the bone inducing material into the proper configuration to fit the site being corrected.

[0003] Many products exist to treat this surgical need. One example is autologous bone particles or segments recovered from the patient. When removed from the patient, it is wet and viscous from the associated blood. This works very well to heal the defect but requires significant secondary surgery resulting in lengthening the surgery, extending

20

the time the patient is under anesthesia and increasing the cost. In addition, a significant increase in patient morbidity is attendant in this technique as the surgeon must take bone from a non-involved site in the patient to recover sufficient healthy bone, marrow and blood to perform the defect filling surgery. This leads to significant post-operative pain.

[0004] Another product group involves the use of inorganic materials to provide a matrix for new bone to grow at the surgical site. These inorganic materials include hydroxyapatite obtained from sea coral or derived synthetically. Either

25

form may be mixed with the patient's blood and/or bone marrow to form a gel or a putty. Calcium sulfate or plaster of Paris may be mixed with water to similarly form a putty. These inorganic materials are osteoconductive but are bioinert and do not absorb or become remodeled into natural bone. They consequently remain in place indefinitely as a brittle, foreign body in the patient's tissue.

[0005] Allograft bone is a logical substitute for autologous bone. It is readily available and precludes the surgical complications and patient morbidity associated with autologous bone as noted above. Allograft bone is essentially a collagen fiber reinforced hydroxyapatite matrix containing active bone morphogenic proteins (BMP) and can be provided in a sterile form. The demineralized form of allograft bone is naturally both osteoinductive and osteoconductive. The demineralized allograft bone tissue is fully incorporated in the patient's tissue by a well established biological mechanism. It has been used for many years in bone surgery to fill the osseous defects previously discussed.

30

35

[0006] It is well known in the art that for several decades surgeons have used a patient's own blood as a vehicle in which to mix the patient's bone chips or bone powder, or demineralized bone powder so as to form a defect filling paste. Blood is a useful carrier because it is available from the bleeding operative site, is non-immunogenic to the patient and contains bone morphogenic proteins which facilitate wound healing through new bone growth. However, stored blood from other patients has the deficiencies that any blood transfusion would have such as blood type compatibility, possibility of transmission of disease and unknown concentration of BMP which are to a great extent dependent upon the age of the donor.

40

[0007] While blood contains from forty percent (40%) to fifty percent (50%) cell mass, it is a satisfactory carrier for demineralized bone powder because it contains both mono- and polysaccharides which contribute to the blood viscosity and provide the bulk viscosity to the paste created by mixing the bone powder and blood. Specific monosaccharides in blood are glucose at a concentration of 60 - 100mg/100ml (0.1%) and polysaccharides such as hexose and glucosamine at approximately 0.1%. Glucuronic acid is also present at approximately 0.4 - 1.4mg/100ml (average 0.01%).

45

[0008] The problems inherent with using the patient's blood as a carrier for demineralized bone powder are the difficulties of mixing the same at the operating site, the difficulty in obtaining a bone paste consistency which can be easily applied to the surgical area, the guesswork in mixing a usable composition at the site and the problem of having

50

a bone paste or gel which will promote optimum bone replacement growth, not be carried away by the body fluids at the operation site or simply fall out of the bone defect site. In an attempt to solve these and other problems, there have been a number of other attempts using other alternative mixtures and compositions.

[0009] Demineralized allograft bone is usually available in a lyophilized or freeze dried and sterile form to provide for extended shelf life. The bone in this form is usually very coarse and dry and is difficult to manipulate by the surgeon. One solution to use such freeze dried bone has been provided in the form of a gel, GRAFTON®, a registered trademark of Osteotech Inc., which is a simple mixture of glycerol and lyophilized, demineralized bone powder of a particle size in the range of 0.1 cm to 1.2 cm (1000 microns to 12,000 microns) as is disclosed in U.S. Patent Number 5,073,373.

55

[0010] GRAFTON works well to allow the surgeon to place the allograft bone material at the site. However, the carrier,

glycerol has a very low molecular weight (92 Daltons) and is very soluble in water, the primary component of the blood which flows at the surgical site. Glycerol also experiences a marked reduction in viscosity when its temperature rises from room temperature (typically 22° C in an operating room) to the temperature of the patient's tissue, typically 37°C. This combination of high water solubility and reduced viscosity causes the allograft bone material to be "runny" and to flow away from the site almost immediately after placement; this prevents the proper retention of the bone within the site as carefully placed by the surgeon.

[0011] These problems with GRAFTON gel have been attempted to be resolved by using a much larger particle size of allograft bone, specifically lamellae or slivers of bone created by milling or slicing the bone before mixing it with the glycerol carrier. This improves both the bulk viscosity and the handling characteristics of the mixture but still leaves the problem of the fast rate of dissipation of the carrier and some bone due to the solubility of the glycerol carrier. The larger particles of demineralized bone may also retard the development of new bone by the patient because the large bony lamellae do not pack as well as the smaller grainy particles of bone. This will leave more open space and could lengthen the time required to grow new bone and properly fill the defect. Another deficiency of using the bony lamellae is that the ends of the bony fragments are uneven and when packed into the surgical defect, leave uneven filaments of bone protruding out from the defect which can compromise the healing rate.

[0012] U.S. Patent No. 5,290,558 discloses a flowable demineralized bone powder composition using an osteogenic bone powder with large particle size ranging from about 0.1 to about 1.2 cm. mixed with a low molecular weight polyhydroxy compound possessing from 2 to about 18 carbons including a number of classes of different compounds such as monosaccharides, disaccharides, water dispersible oligosaccharides and polysaccharides.

[0013] Hence, the advantages of using the smaller bone particle sizes as disclosed in the 5,073,373 gel patent were compromised by using bone lamellae in the shape of threads or filaments and retaining the low molecular weight glycerol carrier. This later prior art is disclosed in U.S. Patent Numbers 5,314,476 and 5,507,813 and the tissue forms described in these patents are known commercially as the GRAFTON Putty and Flex, respectively.

[0014] The use of the very low molecular weight glycerol carrier also requires a very high concentration of glycerol to be used to achieve the bulk viscosity. Glycerol and other similar low molecular weight organic solvents are toxic and irritating to the surrounding tissues. Furthermore glycerol has been reported to be specifically neurotoxic and this problem is compounded when the concentration of glycerol is at the 20 - 95% level as disclosed in the 5,073,373 patent.

[0015] Another attempt to solve the bone composition problem is shown in U. S. Patent No. 4,172,128 which discloses demineralized bone material mixed with a carrier to reconstruct tooth or bone material by adding a mucopolysaccharide to a mineralized bone colloidal material. The composition is formed from a demineralized coarsely ground bone material, which may be derived from human bones and teeth, dissolved in a solvent forming a colloidal solution to which is added a physiologically inert polyhydroxy compound such as mucopolysaccharide or polyuronic acid in an amount which causes orientation when hydrogen ions or polyvalent metal ions are added to form a gel. The gel will be flowable at elevated temperatures above 35°C and will solidify when brought down to body temperature. Example 25 of the patent notes that mucopolysaccharides produce pronounced ionotropic effects and that hyaluronic acid is particularly responsible for spatial cross-linking. Unfortunately this bone gel is difficult to manufacture and requires a premolded gel form.

[0016] U.S. Patent No. 4,191,747 teaches a bone defect treatment with coarsely ground, denatured bone meal freed from fat and ground into powder. The bone meal is mixed with a polysaccharide in a solution of saline and applied to the bone defect site.

[0017] Another prior art product is the formulation of demineralized allograft bone particles in collagen. Both bovine and human collagen have been used for this application. Bovine collagen carries the risk of an immunogenic reaction by the recipient patient. Recently, it has been found that a disease of cattle, bovine spongiform encephalopathy (BSE) is transmitted from bovine tissue to humans. Thus, bovine tissue carries a risk of disease transmission and is not a desirable carrier for allograft tissue.

[0018] Human collagen is free of these animal based diseases. However, collagen absorbs slowly in the human body, particularly in a bony site with usually a low degree of vascularity. The slow absorption of collagen can delay the growth of new bone and result in the formation of scar tissue at the site. This could result in a non-bony healing and a result with much less tensile strength.

[0019] Accordingly, the prior art as embodied in the glycerol and other carrier based technology to deliver demineralized allograft bone to a surgical osseous site is replete with problems and only partially addresses the problems inherent in the correcting surgical defects.

SUMMARY OF THE INVENTION

[0020] A bone putty with a useful bulk viscosity has been achieved by using a very high molecular weight class of soluble biomaterial, hydrogel. The use of high molecular weight hydrogels preferably over one million Daltons allows the achievement of a very malleable bone putty with only 1 - 5% concentration of the hydrogel in the carrier. The carrier

formulation is an aqueous solution, preferably including a sodium chloride based phosphate buffer which avoids the toxic problems with the high concentrations of the low molecular weight organic solvents of the prior art.

[0021] It can thus be seen that the prior art has attempted to replicate putty/gel obtained by the mixing of blood with bone particles without the necessity of mixing the two together at the surgical site in non-controlled proportions and under time and space prohibitions.

[0022] The selection of high molecular weight hydrogels allows the use of the preferred small particle size granules of demineralized allograft bone. These small particles pack better in the wound defect and absorb more quickly thereby allowing the bone defect to be remodeled into the natural bone of the patient.

[0023] It is an object of the invention to utilize demineralized powdered bone in a particle size that is useful to achieve the malleability characteristics that maximizes the amount of bone in the formulation without creating a gritty, less malleable characteristic.

[0024] It is an additional object of the invention to use a non toxic carrier for the bone particles which will not adversely impact on the patient.

[0025] It is also an object of the invention to create a bone defect material which can be easily handled by the physician and does not degenerate when contacting blood flow at the surgical site.

[0026] In some embodiments of the invention a calcium salt is used with the demineralized bone composition to aid in healing at the bone defect site.

[0027] In other embodiments the invention provides a premixed bone putty/gel in an oxygen protected carrier to keep the putty/gel from drying out or being degraded.

DESCRIPTION OF THE INVENTION

[0028] The present invention is directed towards a demineralized bone powder composition to heal bone defects. The preferred embodiment of Examples I and VIII are the best mode for the Sodium Hyaluronate putty composition and Examples XV or XVI for the Sodium Hyaluronate gel composition. These and other alternate embodiments of the invention overcome the two basic deficiencies of the glycerol carrier and bone particle flowable compositions used in the prior art: first, the low molecular weight of glycerol; and second, the use of large particle or lamellae to achieve the preferred bulk viscosity. The types of demineralized bone used in the invention are cortical and corticocancellous bone powder.

[0029] Surprisingly, the combination of the 100 - 420 micron particle size of demineralized, lyophilized, allograft bone when mixed with very low concentrations of these very high molecular weight hydrogels in a suitable carrier produces a malleable putty with clinically useful bone inducing properties. The malleable property permits the surgeon to shape the quantity of bone putty or gel to exactly fit the surgical defect. Manipulation of the "lump" of bone putty may be done without it sticking to the gloves of the surgeon, behaving somewhat like a wet clay used in sculpting.

[0030] The ideal carriers for the malleable putty are preferably taken from high molecular weight hydrogels such as 1) Sodium Hyaluronate about 7.0×10^5 - 3.0×10^6 Daltons; 2) Chitosan about 1.0×10^5 - 3.0×10^5 Daltons; 3) Dextran about 1.0×10^3 - 1.0×10^5 Daltons; 4) Pluronic about 7.0×10^3 - 1.8×10^4 Daltons; and 5) N,O-carboxymethylchitosan glucosamine (NOCC) which is an example of the class of hydrogels known as glycosaminoglycan, a hydrogel derivative about 2.0×10^6 - 3.0×10^6 Daltons.

[0031] The molecular weight of the hydrogels used in the carriers set forth in the Examples I-XVIII are: Hyaluronic acid - (1.2×10^6 Daltons), Chitosan - (2.0×10^5 Daltons), Dextran (40,000 Daltons, used in example VII) or the Pluronic block copolymers of polyethylene oxide and polypropylene oxide; Pluronic® F127 - 9849 to 14,600 Daltons (avg. mol. wt.: 12,600 Daltons); Pluronic® F108- 12,700 to 17,400 Daltons (avg. mol. wt.: 14,600 Daltons); N.O.-carboxymethylchitosan glycosaminoglycan hydrogel derivative - 2.0×10^6 - 3.0×10^6 Daltons.

[0032] Demineralized, lyophilized allograft bone of particle size of about 100 to about 420 microns at a concentration of about 30% to 35% w/w is mixed into an isotonic saline solution of 2% hyaluronic acid of an average molecular weight of about 1.2 million Daltons and produces a highly desirable malleable bone putty. Hyaluronic acid is generally described as an acid mucopolysaccharide. It is envisioned that suitable amounts of bone morphogenic proteins (BMP) can be added to either the gel or putty at any stage in the mixing process to induce accelerated healing at the bone site. BMP directs the differentiation of pluripotential mesenchymal cells into osteoprogenitor cells which form osteoblasts. The ability of freeze dried demineralized cortical bone to transfer this bone induction principle using BMP present in the bone is well known in the art. However the amount of BMP varies in the bone depending on the age of the bone donor and the bone processing. Sterilization is an additional problem in processing human bone for medical use as boiling, autoclaving and irradiation over 2.0 mrad is sufficient to destroy or alter the BMP present in the bone matrix.

[0033] After conducting numerous experiments it was found that a gel product with optimal formability and handling properties would have a molecular weight ranging from 690,000 to 1,200,000 Daltons with a Sodium Hyaluronate concentration ranging from 0.75 - 2.0% with a bone concentration ranging from 25 - 27% with a particle size of 100 - 820 microns. This resulted in HA solution viscosities ranging from 1,800 cps to 13,000 cps. It was also found that a

putty product with optimal formability and handling properties would have a molecular weight ranging from 690,000 to 1,200,000 Daltons with a Sodium Hyaluronate concentration ranging from 2.0 - 4.5% with a bone concentration ranging from 30 - 33% with a particle size of 100 - 820 microns. This resulted in HA solution viscosities ranging from 6,000 cps to 275,000 cps. It was found that as the viscosity increases, penetration decreases and when the viscosity is the same the increase in percentage of bone particle weight results in a decrease of penetration.

[0034] Another embodiment of the invention is to induce the presence of soluble calcium at the bone defect site. This will encourage new bone growth through the normal biochemical mechanism. Soluble calcium can be attracted to the surgical site by using a sodium phosphate buffer of pH 6.8 to 7.2 in lieu of the isotonic saline. The phosphate buffer attract calcium cations to the site from the surrounding healthy bone and create an equilibrium concentration of the calcium precisely at the site of healing where it is most desirable to grow new bone.

[0035] Another embodiment of the invention is to create a sponge sheet or sponge mat of bone which is flexible and can be cut to shape by the surgeon. This can be made by using a cross linked hydrogel, either hyaluronic acid or chitosan and suspending a high concentration of bone particles ranging from 250 - 850 microns in size with up to 75% bone by weight. This is then lyophilized or freeze dried to remove the water component via ice sublimation leaving behind a flexible sheet of bone suspended in the dehydrated hydrogel matrix.

[0036] Any number of medically useful substances can be used in the invention by adding the substances to the composition at any steps in the mixing process or directly to the final composition. Such substances include collagen and insoluble collagen derivatives, hydroxy apatite and soluble solids and/or liquids dissolved therein. Also included are antivirals such as those effective against HIV and hepatitis; antimicrobial and/or antibiotics such as erythromycin, bacitracin, neomycin, penicillin, polymyxin B, tetracycline, viomycin, chloromycetin and streptomycin, cefazolin, ampicillin, azactam, tobramycin, clindamycin and gentamycin. It is also envisioned that amino acids, peptides, vitamins, co-factors for protein synthesis; hormones; endocrine tissue or tissue fragments; synthesizers; enzymes such as collagenase, peptidases, oxidases; polymer cell scaffolds with parenchymal cells; angiogenic drugs and polymeric carriers containing such drugs; collagen lattices; biocompatible surface active agents, antigenic agents; cytoskeletal agents; cartilage fragments, living cells such as chondrocytes, bone marrow cells, mesenchymal stem cells, natural extracts, tissue transplants, bioadhesives, transforming growth factor (TGF-beta), insulin-like growth factor (IGF-1); growth hormones such as somatotropin; bone digestors; antitumor agents; fibronectin; cellular attractants and attachment agents; immuno-suppressants; permeation enhancers, e.g. fatty acid esters such as laureate, myristate and stearate monoesters of polyethylene glycol, enamine derivatives, alpha-keto aldehydes can be added to the composition.

[0037] The invention can be further understood by the following examples with the percentages being determined by weight. In some samples a penetration test was used to measure the bulk consistency of the formulation. In principle, the test measures the depth of penetration of a metal cone of a known mass inserted into a sample of the formulation for a fixed time. The heavier a formulation the less penetration occurs. this test is adopted from ASTM Method D 1403-96: Standard Test Method for Cone preparation Lubricating Grease Using One Quarter and One-Half Scale Cone Equipment. All examples could also be done in an aseptic environment to maintain a sterile final product.

Examples of the Invention

[0038] In the examples the molecular weight of the various carrier components used is as follows.

1)	Sodium Hyaluronate	1.2 X 10 ⁶ - 2.6 X 10 ⁶ Daltons
2)	Chitosan	2.0 X 10 ⁵ Daltons
3)	Dextran PM 40	about 40,000 Daltons
4)	Pluronic® F127	9849 to 14600 Daltons (avg. mol. wt.: 12600 Daltons)
5)	Pluronic® F108	12700 to 17400 Daltons (avg. mol. wt.: 14600 Daltons)
6)	NOCC (no example)	N,O-carboxymethyl chitosan glycosaminoglycan hydrogel derivative 2.0 x 10 ⁶ - 3.0 x 10 ⁶ Daltons.

Example 1:

[0039] A malleable putty of 2% solution Hyaluronic Acid in isotonic saline with 250-420 micron cortical allograft bone powder @ 30%.

502 milligrams of freeze dried cortical allograft bone of particle size ranging from 250 - 420 microns was mixed into 1,170 milligrams of a 2% solution of sodium hyaluronate in isotonic saline. The bone component is added to achieve a bone concentration of 30% (w/w). The solution was well mixed and allowed to stand for 2-3 hours at room temperature to provide a malleable putty with excellent formability properties.

Example II:

[0040] A putty of 20% Pluronic F127 with 420-850 micron cortical allograft bone powder @ 50%.

5 519 milligrams of freeze dried cortical allograft bone of particle size of 420-850 microns was mixed into 518 milligrams of a 20% solution of Pluronic F127 in isotonic saline. The bone component is added to achieve a bone concentration of 50%(w/w). The solution was well mixed and allowed to stand for 2-3 hours at room temperature. This provided a putty with poor formability properties.

Example III:

10 [0041] A putty of 20% solution of Pluronic F108 with 420-850 micron cortical allograft bone powder @ 50%.

528 milligrams of freeze dried cortical allograft bone of particle size of 420-850 microns was mixed into 522 milligrams of a 20% solution of Pluronic F108 in isotonic saline. The bone component is added to achieve a bone concentration of 50%(w/w). The solution was well mixed and allowed to stand for 2-3 hours at room temperature. This
15 provided a putty with poor formability properties.

Example IV:

[0042] A malleable putty of 20% solution of Dextran 40PM with 420-850 micron cortical allograft bone powder @ 33%.

20 502 milligrams of freeze dried cortical allograft bone of particle size of 420-850 microns was mixed into 1,024 milligrams of a 20% solution of Dextran 40 PM in isotonic saline. The bone component is added to achieve a bone concentration of 33%(w/w). The solution was well mixed and allowed to stand for 2-3 hours at room temperature. This provided a malleable putty with moderate formability properties.

Example V:

[0043] A malleable putty of 20% solution of Pluronic F127 with 100-300 micron cortical allograft bone powder @ 33%.

503 milligrams of freeze dried cortical allograft bone of particle size of 100-300 microns was mixed into 1,004 milligrams of a 20% solution of Pluronic F127 in isotonic saline. The bone component is added to achieve a bone concentration of 33%(w/w). The solution was well mixed and allowed to stand for 2-3 hours at room temperature. This
30 provided a malleable putty with excellent formability properties.

Example VI:

35 [0044] A malleable putty of 20% solution of Pluronic F108 with 100-300 micron cortical allograft bone powder @ 33%.

502 milligrams of freeze dried cortical allograft bone of particle size of 100-300 microns was mixed into 1,006 milligrams of a 20% solution of Pluronic F108 in isotonic saline. The bone component is added to achieve a bone concentration of 33%(w/w). The solution was well mixed and allowed to stand for 2-3 hours at room temperature. This
40 provided a malleable putty with excellent formability properties

Example VII:

[0045] A malleable putty of 20% solution of Dextran 40 PM with 100-300 micron cortical allograft bone powder @ 33%.

502 milligrams of freeze dried cortical allograft bone of particle size of 100-300 microns was mixed into 1,006 milligrams of a 20% solution of Dextran 40 PM in isotonic saline. The bone component is added to achieve a bone concentration of 33%(w/w). The solution was well mixed and allowed to stand for 2-3 hours at room temperature. This
45 provided a malleable putty with excellent formability properties.

Example VIII:

50 [0046] A malleable putty of 3% solution hyaluronic acid with 100-300 micron cortical allograft bone powder @ 33%.

720 milligrams of freeze dried cortical allograft bone of particle size of 100-300 microns was mixed into 1,402 milligrams of a 3% solution of sodium hyaluronate in an aqueous solution of a sodium chloride based phosphate buffer having a viscosity within the range of from about 230,000 cps to about 275,000 cps. The bone component is added to
55 achieve a bone concentration of 33% (w/w). The solution was well mixed and allowed to stand for 2-3 hours at room temperature. This provided a malleable putty with excellent formability properties.

Example IX:

[0047] A malleable putty of 1% solution hyaluronic acid with 250-420 micron cortical allograft bone powder @ 40%.
605 milligrams of freeze dried cortical allograft bone of particle size of 250-420 microns was mixed into 906
5 milligrams of a 1% solution of sodium hyaluronate in isotonic saline. The bone component was added to achieve a
bone concentration of 40%(w/w). The solution was well mixed and allowed to stand for 2-3 hours at room temperature.
This provided a malleable putty with poor formability properties.

Example X:

[0048] A malleable putty of 3% solution chitosan with 100-300 micron cortical allograft bone powder @ 33%.
507 milligrams of freeze dried cortical allograft bone of particle size of 100-300 microns was mixed into 1,002
10 milligrams of a 3% solution of chitosan in isotonic saline. The bone component is added to achieve a bone concentration
of 33%(w/w). The solution was well mixed and allowed to stand for 2-3 hours at room temperature. This provided a
15 malleable putty with good formability properties.

Example XI:

[0049] A malleable putty of 3% solution chitosan with 420-850 micron cortical allograft bone powder @ 33%.
20 518 milligrams of freeze dried cortical allograft bone of particle size of 420-850 microns was mixed into 1,038
milligrams of a 3% solution of chitosan in isotonic saline. The bone component is added to achieve a bone concentration
of 33%(w/w). The solution was well mixed and allowed to stand for 2-3 hours at room temperature. This provided a
malleable putty with good formability properties.

Example XII:

[0050] A malleable putty of 3% solution chitosan with 420-850 micron cortical allograft bone powder @ 50%.
518 milligrams of freeze dried cortical allograft bone of particle size of 420-850 microns was mixed into 522 mil-
ligrams of a 3% solution of chitosan in isotonic saline. The bone component is added to achieve a bone concentration
30 of 50%(w/w). The solution was well mixed and allowed to stand for 2-3 hours at room temperature. This provided a
malleable putty with poor formability properties.

Example XIII:

[0051] A malleable putty of 3% solution chitosan with 100-300 micron cortical allograft bone powder @ 50%.
518 milligrams of freeze dried cortical allograft bone of particle size of 100-300 microns was mixed into 522
milligrams of a 3% solution of chitosan in isotonic saline. The bone component is added to achieve a bone concentration
of 50%(w/w). The solution was well mixed and allowed to stand for 2-3 hours at room temperature. This provided a
malleable putty with poor formability properties.

Example XIV:

[0052] A flowable gel of 250 - 420 micron particle size cortical allograft bone granules in a 1% solution of Hyaluronic
Acid at a 25% (w/w) of bone content.
45 503 milligrams of allograft freeze dried cortical bone was mixed into 1,502 milligrams of a 1% solution of sodium
hyaluronate having a viscosity within the range of from 2,000 cps to 6,000 cps in an aqueous solution of a sodium
chloride based phosphate buffer. The solution was well mixed and allowed to stand at room temperature to provide a
flowable gel.

Example XV:

[0053] A flowable gel of 250-420 micron particle size cortical allograft granules in a 1% solution of Hyaluronic Acid
at a 30% (w/w) of bone content.
501 milligrams of allograft freeze dried cortical bone was mixed into 1,167 milligrams of a 1% solution of sodium
55 hyaluronate in isotonic saline. The bone component is added to achieve a bone concentration of 30%(w/w). The solution
was well mixed and allowed to stand for 2-3 hours at room temperature. This provided a flowable gel.

Example XVI:

[0054] A flowable gel of 420-850 micron particle size cortical allograft granules in a 1% solution of hyaluronic acid at a 25%(w/w) of bone content.

5 501 milligrams of allograft freeze dried cortical bone was mixed into 1,501 milligrams of a 1% solution of sodium hyaluronate in isotonic saline. The bone component is added to achieve a bone concentration of 25%(w/w). The solution was well mixed and allowed to stand for 2-3 hours at room temperature. This provided a flowable gel.

Example XVII:

10 [0055] A flowable gel of 420-850 micron particle size cortical allograft granules in a 1% solution of hyaluronic acid at a 30%(w/w) of bone content.

500 milligrams of allograft freeze dried cortical bone was mixed into 1,166 milligrams of a 1% solution of sodium hyaluronate in isotonic saline. The bone component is added to achieve a bone concentration of 30%(w/w). The solution
15 was well mixed and allowed to stand for 2-3 hours at room temperature. This provided a flowable gel.

EXAMPLE XVIII: (not shown in chart)

20 [0056] A malleable putty of 2% solution N. O - carboxymethyl chitosan in isotonic saline with 420 - 850 micron cortical allograft bone powder @ 30%.

502 milligrams of freeze dried cortical allograft bone of particle size ranging from 250 - 420 microns was mixed into 1,170 milligrams of a 2% solution of NOCC in isotonic saline. The bone component is added to achieve a bone concentration of 30% (w/w). The solution was well mixed and allowed to stand for 2-3 hours at room temperature to provide a malleable putty with excellent formability properties. Penetration values using the described ASTM method
25 ranged from 74 - 76.

[0057] The following Table I sets forth the above noted examples I - XVII in comparative form:

30

35

40

45

50

55

TABLE I

Example #	Ref #	Carrier Solution	Dose mg/ Carrier mg	Bone %	Particle Size (micron)	Comments	Putty/Gel
I	4.2	2% HA	502 mg; 1170 mg	30	250-120	good	putty; excellent formability
II	1b	20% Pluronic F127	519 mg; 518 mg	50	420-450	too dry; too grainy	putty; poor formability
III	2b	20% Pluronic F108	528 mg; 522 mg	50	420-450	too dry; too grainy	putty; poor formability
IV	3b3	20% Dextran 40 PM	502 mg; 1024 mg	33	420-450	good; grainy; moderate packing capacity	putty; moderate formability
V	1a1	20% Pluronic F127	503 mg; 1004 mg	33	100-300	best; good; keeps shape; very good packing; moldable; sticky	putty; excellent formability
VI	2a2	20% Pluronic F108	502 mg; 1006 mg	33	100-300	best; good but slightly wet; good packing; sticky	putty; excellent formability
VII	3a3	20% Dextran 40 PM	502 mg; 1006 mg	33	100-300	best	putty; excellent formability
VIII	7a7	3% HA	720 mg; 1402 mg	33	100-300	good consistency; slightly sticky and slightly dry	putty; excellent formability
IX	2-6	1% HA	605 mg; 905 mg	40	250-420	too grainy; very dry	putty; poor formability
X	5a5	3% Chitosan	507 mg; 1002 mg	33	100-300	best	putty; good formability
XI	5b5	3% Chitosan	518 mg; 1038 mg	33	420-450	good; grainy; too dry; pellets well; too large granules	putty; good formability
XII	5b	3% Chitosan	518 mg; 522 mg	50	420-450	too dry; too grainy	putty; poor formability
XIII	5a	3% Chitosan	518 mg; 522 mg	50	100-300	too dry; won't hold shape; too dry; not putty-like; too dry; no packing	putty; poor formability
XIV	5-1	1% HA	503 mg; 1502 mg	25	250-420	Wet; still good consistency and formability; very moderately grainy	flowable gel
XV	5-2	1% HA	501 mg; 1167 mg	30	250-420	drier than 5-1; reasonable formability; much grainier	flowable gel
XVI	5-4	1% HA	501 mg; 1501 mg	25	420-450	wet; grainy; not formable; may be flowable	flowable gel
XVII	5-5	1% HA	500 mg; 1166 mg	30	420-450	wet; formable; grainy	flowable gel

In summation, it can be seen from Table I that:

[0058] A flowable gel can be made up of about 25-30% bone powder (particle size in a range of 250-850 microns) mixed into a high molecular weight hydrogel carried in solution, such as 1% sodium hyaluronate (Examples XIV, XV, XVI, XVII).

[0059] A putty with good formability can be made up of about 30-40% of bone powder (particle size in a range of 100-850 microns) mixed into a hydrogel solution, such as a 2-3% sodium hyaluronate or 3% chitosan or a 20% Pluronic

(Examples I, V, VI, VII, VIII, X, and XI).

[0060] Several examples of (II, III, IX, XII, and XIII) of test results are included which did not produce either a successful flowable gel or putty. These show the limits of the concentrations of the respective examples. Particle sizes below about 100 microns will absorb too quickly.

5 [0061] In order to preclude oxidation degradation and loss of viscosity the composition should be mixed and packaged in an oxygen free environment. The mixing of the demineralized bone powder into hydrogel solution is undertaken in an enclosed sterile glove chamber with an oxygen free environment such as in a nitrogen, argon or other inert gas filled chamber. The mixed malleable bone composition is then placed in a sterile container such as an impervious syringe barrel or vial, sealed and placed in a sterile sealed package which is filled with an inert gas or vacuum sealed.

10 [0062] The principles, preferred embodiments and modes of operation of the present invention have been described in the foregoing specification. However, the invention should not be construed as limited to the particular embodiments which have been described above. Instead, the embodiments described here should be regarded as illustrative rather than restrictive. Variations and changes may be made by others without departing from the scope of the present invention as defined by the following claims:

15 Claims

- 20 1. A sterile malleable bone composition for application to a bone defect site to promote new bone growth at the site comprising a mixture of demineralized osteogenic bone powder, in a hydrogel carrier;

the bone powder having a particle size of from about 100 to about 850 microns;
the bone powder comprising from about 25 to about 35% of the weight of the composition;
25 the carrier being selected from the group consisting of sodium hyaluronate, chitosan and N,O-carboxymethyl-chitosan in water solution, the sodium hyaluronate, chitosan or N,O-carboxymethyl-chitosan having a high molecular weight ranging from five hundred thousand to three million Daltons and ranging from 1% to 4.5% by weight of the carrier solution.

- 30 2. A malleable bone composition as claimed in claim 1, wherein said mixture includes bone morphogenic proteins in excess of the amount naturally occurring in allogeneic bone.

3. A malleable bone composition as claimed in claim 1 or 2, wherein the water solution comprises a sodium chloride based phosphate buffer.

- 35 4. A malleable bone composition as claimed in any one of the preceding claims, wherein said bone powder is cortical allograft bone powder or corticocancellous allograft bone powder.

- 40 5. A sterile malleable bone putty composition for application to a bone defect site to promote new bone growth at the site which comprises a new bone growth inducing mixture of demineralized lyophilized allograft bone powder with a particle size ranging from about 100 to about 850 microns in a carrier comprising an aqueous solution of a sodium salt of hyaluronic acid hydrogel, the hyaluronic acid component ranging from 1 to 4.5% by weight of the carrier solution and having a molecular weight of at least 10^6 Daltons and a viscosity ranging from 6,000 to about 275,000 cps, the bone content of the carrier ranging in weight from about 25% to about 35% total weight of the composition.

- 45 6. A malleable bone putty composition as claimed in claim 5 wherein said hydrogel carrier has a 2 - 3% hyaluronic acid concentration with the balance of the carrier formulation containing a sodium phosphate buffer with a pH of 6.8 to 7.2, said buffer attracting calcium and concentrating same at the bone defect site.

- 50 7. A malleable bone putty composition as claimed in claim 5 or 6, including antimicrobial and/or antibiotics such as erythromycin, bacitracin, neomycin, penicillin, polymyxin B, tetracycline, viomycin, chloromycetin and streptomycin, cefazolin, ampicillin, azactam, tobramycin, clindamycin, gentamycin and vitamins.

- 55 8. A malleable bone putty composition for application to a bone defect site to promote new bone growth at the site comprising a new bone growth inducing demineralized lyophilized allograft bone powder with a particle size ranging from about 100 to about 420 microns in a high molecular weight sodium hyaluronate and water hydrogel carrier, the bone content of the composition ranging from about 30% to about 35% by weight and the high molecular weight sodium hyaluronate component ranges from 2% to 4.5% by weight of the carrier has a molecular weight greater

than one million Daltons.

- 5 9. A sterile malleable bone putty composition for application to a bone defect site to promote new bone growth at the site which comprises a new bone growth inducing compound of demineralized lyophilized allograft bone powder with a particle size ranging from about 100 to about 420 microns in a N,O-carboxymethylchitosan and water hydrogel carrier solution, the N,O-carboxymethylchitosan component ranging from 1 to 3% by weight of the carrier weight and having a molecular weight ranging from 2.0×10^6 - 3.0×10^6 Daltons.
- 10 10. A malleable bone putty composition for application to a bone defect site to promote new bone growth at the site which comprises a new bone growth inducing demineralized lyophilized allograft bone powder with a particle size ranging from about 100 to about 850 microns in a high molecular weight chitosan and water hydrogel carrier solution with the bone content of the putty composition ranging from 30% to 35% and the chitosan itosan component comprising about 3% by weight of the carrier solution with the chitosan having a molecular weight ranging from about 1.0×10^3 to 3.0×10^5 Daltons.
- 15 11. A malleable bone gel composition for application to a bone defect site to promote new bone growth at the side which comprises a new bone growth inducing amount of demineralized lyophilized allograft bone powder with a particle size ranging from about 250 to about 850 microns in a high molecular weight hyaluronic acid in water hydrogel carrier with the hyaluronic acid component comprising about 1% of the carrier and having a molecular weight over 1.0×10^6 Daltons, the bone powder content of the composition ranging from about 25% to about 30%.
- 20 12. A malleable bone gel composition for application to a bone defect site to promote new bone growth at the side comprising a new bone growth inducing amount of demineralized lyophilized allograft bone powder with a particle size ranging from about 250 to about 420 microns mixed in a high molecular weight hyaluronic acid and water solution hydrogel carrier with the hyaluronic acid component being present in the amount of about 1% of the carrier and having a viscosity of about 1,800 to 13,000 cps, the bone powder amount content of the composition ranging from about 25% to about 30% by weight.
- 25 13. A sterile malleable bone putty composition for application to a bone defect site to promote new bone growth at the site which comprises a new bone growth inducing compound of demineralized lyophilized allograft bone powder with a particle size ranging from about 100 to about 420 microns in a hydrogel carrier, the hydrogel component ranging from 15% to 25% by weight and having a molecular weight of at least 40,000 Daltons.
- 30 14. A malleable bone putty composition as claimed in claim 13, including the addition of a calcium salt to the hydrogel carrier.
- 35 15. A malleable bone putty composition as claimed in claim 13 or 14 wherein the hydrogel component in said carrier is 20% by weight
- 40 16. A malleable bone putty composition for application to a bone defect site to promote new bone growth at the site which comprises a new bone growth inducing amount of demineralized lyophilized allograft bone powder with a particle size ranging from about 100 to about 420 microns in a high molecular weight hydrogel carrier, the bone amount content of the carrier ranging from about 30% to about 35% and the high molecular weight hydrogel in solution ranges from 2% to 3%.
- 45 17. A composition as claimed in any one of claims 5 to 16, wherein the aqueous solution comprises a sodium chloride based phosphate buffer

50

55



European Patent
Office

EUROPEAN SEARCH REPORT

Application Number
EP 00 30 1370

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
X	WO 99 11298 A (GENSCI REGENERATION LAB INC) 11 March 1999 (1999-03-11) * page 5, line 19 - line 33; claims *	1-17	A61L27/36 A61K35/32 A61L24/00
X	WO 99 52572 A (CHILDRENS MEDICAL CENTER) 21 October 1999 (1999-10-21) * claims *	1-17	
X	WO 98 14222 A (CHILDRENS MEDICAL CENTER) 9 April 1998 (1998-04-09) * claims; examples *	1-17	
X	EP 0 522 569 A (UNITED STATES SURGICAL CORP) 13 January 1993 (1993-01-13) * claims *	1-17	
D,X	US 5 314 476 A (PREWETT ANNAMARIE B ET AL) 24 May 1994 (1994-05-24) * claims; examples 1,2 *	1	
X	US 5 516 532 A (ASHKAR SAMY ET AL) 14 May 1996 (1996-05-14) * claims *	1	TECHNICAL FIELDS SEARCHED (Int.Cl.7) A61L A61K
A	US 5 910 315 A (GERTZMAN ARTHUR A ET AL) 8 June 1999 (1999-06-08) * claims; examples *	1-17	
A	US 5 707 962 A (CHEN CHARLES C ET AL) 13 January 1998 (1998-01-13) * examples *	1-17	
A	EP 0 784 985 A (YAMANOUCHI PHARMA CO LTD) 23 July 1997 (1997-07-23) * claims; examples *	1	
-/--			
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 12 July 2000	Examiner ESPINOSA, M
<p>CATEGORY OF CITED DOCUMENTS</p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document</p>			

EPO FORM 1503 03.82 (P4/C01)



European Patent
Office

EUROPEAN SEARCH REPORT

Application Number
EP 00 30 1370

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
E	US 6 030 635 A (GERTZMAN ARTHUR A ET AL) 29 February 2000 (2000-02-29) * the whole document * -----	1-17	
			TECHNICAL FIELDS SEARCHED (Int.Cl.7)
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 12 July 2000	Examiner ESPINOSA, M
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	

EPPO FORM 1503 03 02 (P04/001)

**ANNEX TO THE EUROPEAN SEARCH REPORT
ON EUROPEAN PATENT APPLICATION NO.**

EP 00 30 1370

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on. The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

12-07-2000

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9911298	A	11-03-1999	AU	9215198 A	22-03-1999
WO 9952572	A	21-10-1999	AU	3387599 A	01-11-1999
WO 9814222	A	09-04-1998	AU	4603197 A	24-04-1998
			EP	0929322 A	21-07-1999
EP 0522569	A	13-01-1993	US	5356629 A	18-10-1994
			CA	2073574 A	13-01-1993
US 5314476	A	24-05-1994	US	5510396 A	23-04-1996
US 5516532	A	14-05-1996	EP	0774981 A	28-05-1997
			WO	9604026 A	15-02-1996
			US	5968556 A	19-10-1999
US 5910315	A	08-06-1999	NONE		
US 5707962	A	13-01-1998	CA	2133253 A	29-03-1996
EP 0784985	A	23-07-1997	AU	3577895 A	26-04-1996
			JP	2986551 B	06-12-1999
			US	5830493 A	03-11-1998
			CA	2200052 A	11-04-1996
			CN	1158573 A	03-09-1997
			WO	9610426 A	11-04-1996
US 6030635	A	29-02-2000	NONE		

EPO FORM P0459

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82